Faculty of Resource Science and Technology

GERMINATION OF *LACTUCA SATIVA* L. SEEDS AFTER STORAGE UNDER DIFFERENT ENVIRONMENTS

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Germination of *Lactuca sativa* L. seeds after storage under different environments

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DECLARATION

I hereby declare that no portion of this project work has been submitted in support of an application for another degree of qualification of this or any other university or institution of higher learning.

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LIST OF ABBREVIATIONS

AOSA	Association of Official Seed Analysts
OSAW	Oriental Science Apparatus Workshops
ISTA	International Seed Testing Association
CRD	Completely Randomized Design
ANOVA	Analysis of Variance
LSD	Least Significant Different
RH	Relative Humidity

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Germination of *Lactuca sativa* L. seeds after storage under different environments

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ABSTRACT

A study was conducted to evaluate the effect of different temperatures on for the storage of *Lactuca sativa* L. seeds. Seeds with 8.75% of moisture content and 59.0% of germination were desiccated with silica gel for 24 hours, and than placed in four different environments: ambient room (28-30˚C; 70-80% RH), refrigerator (3-5˚C; 42% RH), freezer (-4-0˚C; 30-35% RH) and incubator (35-38˚C; 40% RH) for 15 days. Seed stored in refrigerator (3-5˚C; 42% RH) gave high moisture content and the highest germination of 6.16% and 44.0% respectively. Regression analysis indicated the seeds stored in refrigerator (3-5˚C; 42% RH) maintained its germinability up to 109 days. Results showed that storage in refrigerator (3-5˚C; 42% RH) was a suitable condition for *L. sativa* seeds, before used as planting materials.

Key words: *Lactuca sativa* L., critical moisture content, storage, temperature, seed germination.

ABSTRAK

Satu kajian telah dijalankan untuk menilai kesan perubahan suhu penyimpanan yang sesuai bagi biji benih Lactuca sativa L.. *Biji benih dengan kandungan kelembapan 8.75% and kadar percambahan 59.0% telah disikasikan dengan gel silika selama 24 jam, kemudian biji benih ini disimpan di dalam empat persekitaran yang berbeza: suhu bilik (28-30˚C; 70-80% RH), peti sejuk (3-5˚C; 42% RH), peti sejuk beku (-4-0˚C; 30-35% RH) dan inkubator (35-38˚C; 40% RH) dalam jangka masa 15 hari. Biji benih yang disimpan di dalam peti sejuk (3-5˚C; 42% RH) menunjukkan peratus kandungan kelembapan yang tinggi dan percambahan yang paling tinggi iaitu 6.16% dan 44.0%. Analisis regresi menunjukkan penyimpanan biji benih di dalam peti sejuk (3-5˚C; 42% RH) boleh dilanjutkan sehingga 109 hari. Penyimpanan biji benih di dalam persekitaran peti sejuk (3-5˚C; 42% RH) didapati merupakan keadaan yang paling sesuai bagi biji benih L. sativa sebelum digunakan sebagai bahan penanaman.*

Kata kunci: Lactuca sativa L., *kandungan kelembapan kritikal, penyimpanan, suhu, percambahan biji benih.*

CHAPTER 1

INTRODUCTION

1.1 Background

Lactuca sativa L. with common name lettuce or garden lettuce is a leafy annual or biennials herb in the Asteraceae family. It is believed to be one of the first vegetables brought to the new world by explorer Christopher Columbus and has been grown in the United States since colonial times (California Lettuce Research Board as cited in Boriss, 2005). It is originated in the Mediterranean and Near East (Encyclopedia of Life, n. d.). It is a cosmopolitan cultivated plant.

Among the salad crops, *L. sativa* is the most popular vegetable grown on a commercial scale. It is most often grown as a leafy vegetable, but sometimes for its stem and seeds. Vries (1997) has mentioned that the plants generally have a height and spread of 6 to 12 inches which is approximately 15 to 30 centimeter. This is a tall annual leafy herb with a milky juice. It has a root system that includes a main taproot and smaller secondary roots. *L. sativa* flowers more quickly in hot temperatures, while freezing temperatures cause slower growth and sometimes damage to outer leaves (AgriInfo, 2011).

When the plant has past the edible stage, the flower will develop stalks up to 3 feet (0.9 m) high with small yellow blossoms. The capitulate (inflorescences) of *L. sativa* are composed of multiple florets. Each of them has a modified calyx called pappus which becomes the feathery “parachute” of the fruit, a corolla of five petals fused into a ligule and the reproductive parts (Boriss, 2005). The ovaries will form the compressed, obovate (teardrop-shaped) dry fruits that do not open at maturity, measuring 3 to 4 mm long. This fruit have 5 to 7 ribs on each side and are tipped by two rows of small white hairs. The pappus will remains at the top of each fruits as a dispersal structure. Each of the fruit contains only one seed. The seed is approximately 5 mm long and 1 mm width.

Plate 1: The length of *L. sativa* seed about 5 mm

Plate 2: The width of *L. sativa* seed about 1 mm

1.2 Uses and Nutritive Value

In general, *L. sativa* is a popular leaf vegetable. The stalk is also eaten and the seeds can be used for production of oil (Vries, 1997). It is a nutritious leafy vegetable, rich in mineral and a source of vitamin. The energy food value of leaf is low. The vegetable contain vitamin B and C. The seeds contain vitamin E. *L. sativa* is anodyne, sedative, diuretic and expectorant while it seeds are cooling, demulcent and refrignant and the leaves are slightly lypnotic and sedative.

Based on Encyclopedia of Life (n. d.), the composition for the leaves of *L. sativa* of 100 g of food (fresh weight), it has no calories at all with 92.9 % of water, 2.1 g of protein, 0 g of fat, 3 g of carbohydrate, 0.5 g of fibre and 1.2 g of ash. Other compositions that can be found in this plant are minerals and vitamins. 100 g of the plant consists of minerals such as 26 mg of calcium, 30 mg of phosphorus, 0.7 mg of iron, 10 mg of magnesium, 3 mg of sodium and a lot of potassium which is 208 mg per 100 g of food. For the composition of the vitamins, it consists of 2200 mg of vitamin A, 0.4 mg of Niacin and 15 mg of vitamin C in 100 g of the leaves of *L. sativa* (Encyclopedia of Life, n. d.).

1.3 Medicinal Uses

Lactuca sativa is rich in a milky sap that flows freely from any wound. This milky sap hardens and dries when in contact with air. It has a medicinal purpose due to the composition of 'lactucarium' that can be found in this milky sap. Lactucarium is use in medicine for its anodyne, antispasmodic, digestive, diuretic, hypnotic, narcotic and sedative properties (Boriss, 2005).

Besides that, it is also taken internally in the treatment of insomnia, anxiety neuroses, and hyperactivity in children, dry coughs, whooping cough, and rheumatic pain. Based on Vries (1997) studies, the concentration of this compound are low in young plants while the plant which is comes into flower has the most concentrated of that compound. Thus, lactucarium is collected commercially when the plant is flowering.

However, the cultivated *L. sativa* does not contain as much lactucarium as the wild species. This milky sap is collected by cutting the heads of the plants and scraping the juice into china vessels several times a day until the plant is exhausted. Vries (1997) had mentioned that this compound should be used with caution as the different volume of doses can give different effect to the body with normal doses can cause drowsiness , excess causes restlessness and overdoses can cause death through cardiac paralysis.

1.4 Problem Statement

Nowadays, *Lactuca sativa* is commonly demanded especially in the food industry as the part of the ingredients in the cooking which are the most widely used salad crop or as a decoration in the dishes itself (Encyclopedia of Life, n. d.). Since it has high commercial values, the storage of the seeds is necessary in order to meet the demand in the market. However, the storage of the *L. sativa* seeds is rarely done (AgriInfo, 2011). Thus, this study was conduct to access environmental factors of different temperatures suitable for the storage of *L. sativa* seeds, prior to use as a planting material.

1.5 Objectives

The main objectives of this study were:

i. To analyze the influence of different storage environments based on different temperatures and relative humidities on germination of *L. sativa* seeds.
ii. To determine the suitable environmental conditions for storage of *L. sativa* seeds use as a planting material.

CHAPTER 2

LITERATURE REVIEW

2.1 Moisture Content

The seed moisture content is defined as the amount of water in seed and usually expressed by percentage. Agrawal (1993) mentioned that the moisture content is either the loss in weight when the seed is dried or the quantity of water collected when it is distilled. It also becomes one of the most important factors that will influence the retention of viability and the general appearance of seeds. The rules regarding seed moisture does not apply above 14 or below 5% seed moisture (Copeland & McDonald, 1995). Harrington (1960) reported that below 4 to 6% seed moisture content lipid autoxidation becomes a destructive factor and seeds become more susceptible to mechanical damage.

The seed moisture content of about 6 to 8% is optimum for storage of most crop species for maximum longevity (Elias *et al.*, 2006). Seeds stored at moisture contents above 14% begin to exhibit increased respiration, heating, and fungal invasion that destroy seed viability more rapidly than that indicated by the first rule of thumb which is every one percent in seed moisture content decrease, the life of seed will be doubled and this is again hold good between 4 to 12˚C (Harrington, 1960). Copeland and McDonald (1995) mentioned that in the open storage, the moisture content of the seeds will change as a result of the equilibration of the moisture content of the seeds and atmosphere and the result is different when seeds are stored accordingly to the different environment. There are a few methods in determine the moisture content of seeds, such as drying without heat, Lyophilization (Freeze dried), reversibility method and air oven method (Agrawal, 1993).

Air ovens are the simplest method since it is more convenient and less expensive than other drying methods (Chen, 2003). Universal OSAW Moisture meter which is the equipment that measures the moisture content of the seed also can be used in moisture content determination that based on the principle of the electrical conductivity of a moist material which is directly proportional to the amount of moisture contained in it and on the temperature.

2.2 Seed Germination

Seed germination is the most fascinating areas of plant growth and development and a complex process involving many individual reactions and phases, each of which is affected by temperature. For the seed to be germinated, it must be subjected to the appropriate environmental conditions such as available water, a proper temperature range, a supply of oxygen, and sometimes light (Copeland *et al.*, 1995). Changing oxygen, light, temperature, and / or humidity each may play a role in triggering germination (Mohammad *et al*., 2011).

Some seeds will not germinate until they have undergone a process of after-ripening, defined as metabolic changes that must take place in the seed in order for it to overcome dormancy (Li *et al.*, 2012). Some researchers further explained that germination is the activation of metabolic activity of the embryo leading to the emergence of a new seedling plant (Agrawal, 1993). There are two methods that are commonly used to evaluate the quality of seeds after each treatment and these are moisture content and germination tests. The germination test also used to determine seed viability.

AOSA (1991) stated that germination is the emergence and development from the seed embryo of those essential structures that are indicative of the ability to produce a normal plant under favorable condition. Temperature affects both germination percentage and germination speed. There are three temperature points which is minimum, optimum, and maximum that is varying with the species, usually designated for seed germination.

2.3 Seed Viability

Seed viability is denoted as a seed that contains structures and substances including the enzymes systems which give it the capacity to germinate under favorable conditions in the absence of dormancy (Copeland *et al.*, 1995). Tetrazolium test will give the percentage of viable embryos and is recognized as an accurate means of estimating embryo viability.

Tetrazolium is a biochemical compound (2, 3, 5-triphenyl tetrazolium chloride) that is used for assessing the quality of seeds, embryos or tissues. Tetrazolium functionally estimates the viability, assessing vigour and diagnosing physiological problems in particular seeds, embryos or tissues.

Seed viability is highest when the seeds are at physiological maturity and viability will gradually decline after reaching physiological maturity (Lee, 2005). The viability would need to be determined at the start of storage and at regular intervals during storage to predict the correct time for regeneration of the accession (Hanson, 1985).

2.4 Seed Dormancy

Seed dormancy is the viable seeds that fail to germinate when provided with the specified germination conditions for the kind of seed. Finch-Savage and Leubner-Metzger (2006) noted that the seed dormancy is an innate seed property which is the environmental conditions that affected the seed to germinate.

Dormancy is often a difficult response to measure but three major categories have been proposed which is exogenous dormancy, endogenous dormancy and secondary or ecodormancy (AOSA, 1985). Exogenous dormancy is the factors outside of the embryo control dormancy (seed coat), endogenous dormancy is the factors associated with the embryo control or mediate while ecodormancy is imposed by environmental factors.

2.5 Seed Deterioration

Elias *et al*. (2006) have mentioned that the rate of seed deterioration can be slowed down by a good storage environment. Seed deterioration is inexorable and irreversible process, the physiological quality of the seed cannot be stored once it deteriorates. In addition, if the seeds are exposed to prolonged rain before threshing, it can contribute to a faster rate of deterioration and also fungal invasion which is the cracks in seeds serve as entrance to pathogens causing subsequent deterioration (Arulnandhy & Senanayake, 1986).

Meanwhile, the seeds that have been developed in a plant growing under environmental stress conditions such as nutrient deficiency, drought, and extreme temperature can become more susceptible to rapid deterioration in storage (Elias *et al.*, 2006). A seed is at its maximum potential vigor when it is at maturation stages; thereafter its quality begins to decline. This matured seeds would deteriorate and lose their vigor if left on the plants in the field and exposed to prolonged wet humid weather prior to harvesting (Heydecker, 1972).

The effective means of maintaining seed quality in storage is by decreasing temperature and seed moisture (Harrington, 1960). The influence of temperature and relative humidity on seed deterioration is exerted right from storage through the development of seedlings and mature plants. Harrington (1960) has stated that the high quality seeds are susceptible to deterioration when stored under adversed conditions of high temperature and relative humidity, rendering them worthless for planting after period of storage.

2.6 Seed Storage

The duration of the successful seed storage depends upon both the aims and the species concerned. Seeds of species with orthodox seed storage behavior can be maintained satisfactorily ex situ over long term in appropriate environments (Hong & Ellis, 1996). However, the absolute longevity of seed accessions can differ markedly among species even in the same environment.

Hong and Ellis (1996) stated that it is important to identify whether the species shows orthodox, intermediate or recalcitrant seed storage behavior in order to determine the most suitable storage environment(s) and duration of successful storage. A factor for good seed storage is when dry seed are kept in containers that allow free movement of moisture, with low humidity during the storage period will remain the seed at low moisture content (Agrawal, 1993).